RESPIRATORY TRACT ILLNESS SURVEILLANCE IN PATIENTS AT A COMMUNITY CLINIC DURING THE 2010 INFLUENZA SEASON

by

Annette Atkinson

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STATEMENT OF THESIS APPROVAL

The thesis of Annette Atkinson

has been approved by the following supervisory committee members:

Lisa Gren , Chair 10/23/2012
Date Approved

John Kriesel , Member 10/22/2012
Date Approved

Joseph Lynn Lyon , Member 10/23/2012
Date Approved

and by Peter E. Jensen , Chair of

the Department of Pathology

and by Charles A. Wight, Dean of The Graduate School.

ABSTRACT

Viruses cause the majority of respiratory infections. A rapid laboratory method to accurately diagnose etiology of respiratory illness can inform physicians and guide treatment decisions. The objective of this cross-sectional, laboratory based surveillance study is to assess the sensitivity and specificity of influenza virus rapid antigen assay Quidel A+B (Quidel, San Diego, CA) and two multiplex respiratory pathogen assays [Idaho Technology FilmArray® Respiratory Panel (Idaho Technology, Inc., Salt Lake City, UT) and Qiagen ResPlex™ II (Qiagen, Germantown, MD)] compared with influenza virus polymerase chain reaction (PCR) assays.

Patients seeking treatment for influenza-like-illness (ILI) in the outpatient setting were identified between October 17, 2010 and May 31, 2011. A total of 1,481 ILI patients were seen. Nasal specimens were collected and tested on all assays for 253 of these patients. Sensitivity and specificity of each influenza and multiplex respiratory assay was calculated relative to a gold standard PCR assay. The self-reported symptom profiles of each organism identified in the respiratory pathogen panels were compared.

The median patient age was 12 years (range: 0-89 years); 176 (70%) had one or more viruses detected in the nasal swab specimen. Sensitivity for influenza A was 92% (95% CI: 88-99%) for FilmArray® RP, 85% (95% CI: 81-96%) for ResPlex™ II, and 38% (95% CI: 48-71%) for Rapid, with specificities of

99.5-100%. Sensitivity for influenza B was 85% (95% CI: 86-99%), 70% (95% CI: 66-90%) for ResPlex™ II, and 6% (95% CI: 0-23%) for Rapid, with specificities of 99.5-100%. A similar pattern was seen for noninfluenza viruses, with the FilmArray® RP assay being more sensitive and specific than the ResPlex™ II. The use of multiplex viral assays is becoming more common in point-of-care settings. In this study, the Idaho Technology FilmArray® Respiratory Panel was more sensitive and specific than the Qiagen ResPlex™ II assay for influenza A and B viruses, as well as other common respiratory viruses.

In dedication to my parents for their continued support and guidance.

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INTRODUCTION

Viral infections cause the majority of respiratory tract illness. Clinical symptoms alone do not accurately diagnose the etiologic agent of infection. Inappropriate use of antibiotics for viral infections has led to health care guidelines with respiratory tract illness algorithms[1] to reduce the use of antibiotics for treatment of viral illness. While the appropriate use of antivirals can reduce the duration and severity of illness[2] they are only effective for influenza infections. Rapid, accurate laboratory testing can guide diagnosis and treatment decisions in the outpatient setting.

Traditional syndromic surveillance[3] has been used to identify illness trends in the community to guide health care provider treatment decisions. Syndromic surveillance by the University of Utah Primary Care Research Network (UUPCRN) is provided electronically to the Utah Department of Health (UDOH). The data sent by UUPCRN are a weekly composite of influenza-like-illness (ILI) patient visits by the following age groups: <1 year, 1-4 years, 5-17 years, 18-24 years, 25-64 years, and ≥65 years. UUPCRN's data are merged with that provided by other sentinel providers throughout the state, and the aggregated data are published weekly[4] to inform healthcare providers about the illness trends experienced in the community.

The evolution of point-of-care testing [rapid and now polymerase chain reaction (PCR)] can provide more immediate patient benefit. This study is an

extension of a laboratory-based surveillance study (using additional assays) designed to identify the causal agent associated with ILI in the outpatient setting during the 2010-11 influenza season. This study was part of an Influenza Incidence Pilot (IIP) project by the Centers for Disease Control and Prevention (CDC) and the UDOH. The IIP project was funded by the CDC and the Council of State and Territorial Epidemiologists (CSTE) for the 2010-11 influenza season at 12 sites in the United States. In Utah, a contract was awarded to the UDOH, with a subcontract to UUPCRN. Patients with ILI who were seen at the UUPCRN Urgent Care or the Redwood outpatient clinic were eligible for the study. Study specimens were tested at the Unified State Laboratory: Public Health (USLPH), the public health laboratory for the UDOH located in Taylorsville, Utah. We evaluated the diagnostic characteristics of several potential point-of-care tests.

For the IIP study, all samples were tested with a point-of-care, rapid antigen test for influenza A and B (Quidel A+B, Quidel, San Diego, CA), as well as with the CDC Human Influenza Virus Real-time (RT-PCR) Detection and Characterization Panel (CDC, Atlanta, GA), an influenza virus PCR assay. All samples were further tested using a multiplex respiratory virus panel (Qiagen ResPlex™ II, Qiagen, Germantown, MD) to determine the associated etiologic agent. This report is a study using the IIP samples to evaluate the sensitivity and specificity, relative to PCR, of a new point-of-care test for respiratory viral pathogens. Residual specimens from the IIP study were evaluated on the FilmArray® Respiratory Panel (RP) (Idaho Technology, Inc., Salt Lake City, UT), and the outcomes were compared with those from the Qiagen ResPlex™ II assay. At the time of the study the FilmArray® RP kits were Research Use Only (RUO),

and were approved by the United States Food and Drug Administration (FDA) in May 2012. The Resplex™ II kit is not FDA approved and was used as a RUO assay.

METHODS

Patient Eligibility and Sample Collection

The UUPCRN has 10 outpatient clinics that provide continuity care, and an associated Urgent Care Clinic that operates after hours and on weekends. These clinics are located in the Salt Lake City, Utah metropolitan area. There are about 120,000 patients who are seen within this clinic system for a total of 350,000 annual visits. Between October 17, 2010 and May 31, 2011, eligible patients were those seen for ILI at the Urgent Care or by 2 providers at the Redwood Clinic, located in Salt Lake City, Utah. The 2 providers were selected for the IIP study because they saw a larger proportion of acutely ill patients within the Redwood Clinic. One was a physician board-certified in Internal Medicine and Pediatrics, while the other was a Nurse Practitioner in the Family Medicine clinic. The maximum number of study patients from whom samples were collected each week was 50: the first 30 patients at the Urgent care and the first 10 patients of each of the 2 providers. This study was approved by the University of Utah (#37465) and the Utah Department of Health Institutional Review Boards (IRB).

For patients older than 2 years, ILI was defined as presence or history of a fever of 100°F or higher accompanied by either cough or sore throat in the absence of a known, alternative cause of the symptoms. Because young children may not be able to express symptoms such as sore throat, the ILI definition for those age 2 years or younger included presence or history of a fever of 100°F

with any associated respiratory symptom, such as cough, sore throat, or rhinitis, that was absent another known cause. Patients or their parents were asked at visit check-in to complete a brief survey that identified their history of fever and respiratory symptoms. The medical assistants used this form to identify patients who were eligible for the study, obtain verbal consent, and collect a nasal swab specimen. The nasal swab specimens were placed into Remel M5™ (Remel, Lenexa, KS) viral transport medium and stored in the clinic laboratory refrigerator prior to transport on the same or the next business day. A courier transported the specimens on ice packs to USLPH, where the Quidel rapid assay and CDC RT-PCR assay for influenza were completed within 7 days. Samples were then aliquoted and frozen until the end of the study, when the ResPlex™ II and FilmArray© RP assays were completed on thawed samples. Samples that had discrepant results on the multiplex assays were sent for confirmatory PCR testing at CDC (for picornaviruses, coronavirus, and parainfluenza viruses) or Southern Nevada Public Health Laboratory (for adenovirus, human metapneumovirus, and respiratory syncytial virus). Patient demographics, such as age, sex, and zip code were abstracted from the electronic medical record (EMR).

Laboratory Tests

Refrigerated specimens were tested with the Quidel A+B rapid influenza antigen test within 1 week of being collected. When rapid influenza testing was completed, the specimens were accessioned into the USLPH Influenza testing workflow. Viral ribonucleic acid (RNA) was extracted with the QIAamp Viral RNA Mini kit[5] (Qiagen, Germantown, MD) on the Qiagen QIAcube. The extracted RNA

was tested for Influenza A and B by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) on the Applied Biosystems™ 7500 Fast Dx Real Time PCR instrument (Applied Biosystems, Foster City, CA), according to the CDC Human Influenza Virus Real-time (RT-PCR) Detection and Characterization Panel[6] package insert. Samples that were positive for influenza A were also subtyped as seasonal H1, seasonal H3, or pandemic (PDM) H1 2009.[7] When the Influenza PCR testing was complete, the specimens were aliquoted (400µL/vial) and frozen at -80°C. The weekly PCR data were sent to the UDOH epidemiologists to be matched with EMR demographic and self-reported symptom data provided by UUPCRN, and sent to the Council of State and Territorial Epidemiologists (CSTE).

The Qiagen ResPlex™ II, version 2.0[8] Research Use Only (RUO) Panel, was used to test the study specimens (see Table 1 for assay-specific pathogens). A specimen aliquot was thawed and nucleic acid extracted using the MagnaPure Compact Total Nucleic Acid Kit I[9] (Roche Applied Sciences, Mannheim, Germany) on the Roche MagnaPure Compact instrument. The ResPlex™ II primers were used to perform PCR on an Applied Biosystems™ GeneAmp 9700 PCR system (Applied Biosystems, Foster City, CA). The PCR product was then hybridized to beads according to the ResPlex™ II handbook, and analyzed on the Luminex® 100 IS system (Luminex, Austin, TX) using the QIAplex MDD software (Qiagen, Germantown, MD). The positive cut-off threshold value for each virus analyte was calculated for each instrument batch by multiplying the average of the 3 negative controls by 6.[10] The specimen was considered positive if the mean flourescence intensity (MFI) value was greater than the positive cut-off threshold

value for the individual virus analyte. The ResPlex™ II data were sent to UDOH to be matched to EMR demographic and self-reported symptom data and assay results were sent to CSTE in June 2011.

The specimens were tested with Idaho Technology's FilmArray® Respiratory Pathogen Panel[11] (RP); see Table 1 for assay specific pathogens. The FilmArray® RP uses a pouch system that contains all needed reagents for specimen extraction and PCR on the FilmArray® instrument.[12] Nasopharyngeal specimens are the specimen source approved by the FDA; the sample type in this study (nasal swabs) have not been validated by FDA on the FilmArray® RP assay.

Table 1. Multiplex respiratory pathogen assay comparison

Pathogen	FilmArray® RP	Resplex™ II
Adenovirus	Yes	Yes
Bocavirus	Yes*	Yes
Bordetella pertussis	Yes	No
Chlamydophila pneumoniae	Yes	No
Coronavirus	Yes	Yes
Human Metapneumovirus	Yes	Yes
Influenza A	Yes	Yes
Influenza B	Yes	Yes
Mycoplasma pneumoniae	Yes	No
Parainfluenza	Yes	Yes
Picornaviruses	Yes	Yes
Respiratory Syncytial Virus	Yes	Yes
*Research Use Only Panel		

The results for the specimens tested on the ResPlex™ II assay and the FilmArray® Respiratory Pathogen panel were compared. All discrepant samples were sent to another laboratory for testing by PCR to resolve the differences. The Southern Nevada Public Health laboratory tested for adenovirus, human metapneumovirus, and respiratory syncytial virus; Dr. Dean Erdman's laboratory at the CDC tested for picornaviruses, coronavirus, and parainfluenza viruses.

The gold standard (defined in Table 2) for influenza and noninfluenza viruses was used to compute test characteristics. The gold standard for influenza viruses was the ULSPH PCR result. The gold standard for the noninfluenza viruses was determined by a combination of results: either agreement between the ResPlex™ II and FilmArray® RP, or agreement between the confirmatory PCR and one of the multiplex assays.

Table 2. Definition of gold standard

Viruses	True Positive	True Negative
Influenza Viruses	Positive PCR at USLPH	Negative PCR at USLPH
Noninfluenza Viruses	Positive by ResPlex™ and FilmArray® or Positive by confirmatory PCR and one multiplex assay	Negative by ResPlex™ and FilmArray® or Negative by confirmatory PCR and one multiplex assay

Statistical Analysis

The picornaviruses were grouped together for comparisons among viruses since the FilmArray® RP does not differentiate between rhinovirus and enterovirus and the ResPlex™ II rhinovirus and coxsackievirus/enterovirus targets cross react,[13] making it difficult to distinguish between rhinovirus and enterovirus. Specimens positive for more than one virus were not included in the laboratory symptom figures since there was no way to determine which virus caused the patient symptoms. The laboratory results for adenovirus, human metapneumovirus, and parainfluenza virus are not displayed in the symptom figures since the number detected for each virus was less than 10.

Demographics for patients who provided nasal samples were compared with those of all patients meeting the ILI case definition in the participating clinics. Differences between the groups were evaluated by Chi Square using VassarStats: Website for Statistical Computation.[14]

The results for the Quidel A+B rapid influenza antigen test, Qiagen ResPlex™ II, and Idaho Technology FilmArray® RP were compared to the gold standard (PCR) to compute test characteristics, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR). The concordances, expressed as kappa with 95% confidence intervals (CI), were calculated using VassarStats: Website for Statistical Computation.[15]

Prior to being seen by a clinical provider, patients self-reported their symptoms for the IIP study: abdominal pain, anorexia, chills, cough, diarrhea,

earache, headache, malaise, myalgia, nasal congestion, rhinorrhea, and vomiting. We created 2 aggregate groupings: 1 for other respiratory symptoms (rhinorrhea, nasal congestion, earache), and 1 for abdominal symptoms (abdominal pain, diarrhea, vomiting). The proportion of each self-reported symptom or aggregate group was tabulated by virus positivity (based on the result from the multiplex testing). For each symptom or symptom grouping, the Marascuillo procedure[16] was used to test for the equality of symptom proportions across viruses, using Excel (Mac 2008, version 12.3.3, Microsoft Corporation).

RESULTS

Patients

The total number of patients with ILI seen at the Urgent Care clinic or by the 2 primary care physicians participating in the study was 1,481 (patient demographics listed in Table 3); the patient ages ranged from 0.11 to 89 years, with a median age of 9 years. A total of 265 patient specimens were collected; the patient ages ranged from 0.3 to 70 years, with a median age of 12 years. Twelve patients were dropped from the analysis because their specimens had insufficient volume, had no human cells present, or sample quality was unsatisfactory. This left 253 samples from patients for analysis.

Table 3. Patient demographics

Age Range (years)	ILI database (1,481)	Study subjects (253)
<5	34%	27%
5 - 9	18%	17%
10 -19	18%	20%
20 - 49	24%	32%
50 and older	5%	4%
Median*	9 years	12 years
Youngest	0.11 years	0.3 years
Oldest	89 years	70 years
Males	46%	47%

*No statistical difference

The IIP study subjects comprised 17% of the ILI database. The following age ranges, as specified in the IIP protocol, were compared to each other: <5 years, 5-9 years, 10-19 years, 20-49 years, and 50 years and older. The proportion of males to females was similar with males comprising 47% of the study patients. No statistically significant differences were found between the IIP and the ILI patients, for either median age or gender.

Laboratory Data

Test characteristics for both influenza A and B were more sensitive and specific for FilmArray® RP, followed by ResPlex™II, and then rapid antigen (Table 4). For influenza A, the FilmArray® RP had 92% sensitivity, 99.5% specificity, and positive and negative predictive values of 98%, with a kappa of 0.94. For influenza B, the FilmArray® RP had 85% sensitivity, 100% specificity, and positive and negative predictive values of 100% and 98%, respectively; with a kappa of 0.93. The positive likelihood ratio (PLR = sensitivity/proportion false positives) estimates the relative probability of a positive test result in those with the disease compared to those without the disease. Negative likelihood ratios (NLR = proportion false negative/specificity) were also calculated. Additionally, the FilmArray® RP was able to subtype influenza A viruses; the sensitivity for influenza A subtypes ranged from 90 - 93%.

Although a positive result for influenza with a rapid test can be considered a true positive, a negative rapid result for influenza in a symptomatic patient cannot be assumed to be a true result.[17] The positive and negative likelihood

Table 4. Influenza virus test characteristics for 253 samples tested by FilmArray® RP, ResPlex™ II, and Rapid antigen compared with rRT-PCR

Viruses	Method	Gold Standard (+)	Gold Standard (-)	Sens.	Spec.	PPV	NPV	PLR	NLR	Kappa (95% CI)
Influenza A	FilmArray® (+)	48	1	92%	99.5%	98%	98%	186	0.08	0.94 (0.88-0.99)
	FilmArray® (-)	4	200							
	ResPlex™ (+)	44	1	85%	99.5%	98%	96%	170	0.15	0.88 (0.81-0.96)
	ResPlex™ (-)	8	200							
	Rapid (+)	20	0	38%	100%	100%	64%	UTC	0.62	0.59 (0.48-0.71)
	Rapid (-)	32	201							
Influenza B	FilmArray® (+)	28	0	85%	100%	100%	98%	UTC	0.15	0.93 (0.86-0.99)
	FilmArray® (-)	5	220							
	ResPlex™ (+)	23	1	70%	99.5%	96%	96%	152	0.30	0.78 (0.66-0.90)
	ResPlex™ (-)	10	219							
	Rapid (+)	2	0	6%	100%	100%	88%	UTC	0.94	0.10 (0-0.23)
	Rapid (-)	31	220							

Sens. - sensitivity
Spec. - specificity
PPV - positive predictive value
NPV - negative predictive value
PLR - positive likelihood ratio
NLR - negative likelihood ratio
UTC - unable to calculate due to zero in denominator

ratios are used to determine the likelihood of having the disease, given a positive or negative test result, respectively. Generally, likelihood ratios near 1 indicate the test is uninformative in making the diagnosis, while increasingly extreme values suggest a higher probability of having (for PLR) or not having (for NLR) the disease. These ratios are provided in Table 4, where UTC indicates that the ratio could not be calculated because the denominator was zero.

The noninfluenza viruses detectable by both the FilmArray® RP and the ResPlex™ II test characteristics are listed in Table 5. For all viruses, the FilmArray® RP had better test characteristics than the ResPlex™ II. Test characteristics were fairly similar for human metapneumovirus and parainfluenza, but the FilmArray® RP was substantially better for the remaining viruses.

Table 5. Noninfluenza viruses comparison of positive and negative results by FilmArray® RP, ResPlex™ II, and PCR for viruses detectable by both assays

Viruses	Method	Gold Standard (+)	Gold Standard (-)	Sens.	Spec.	PPV	NPV	PLR	NLR	Kappa (95% CI)
Adenovirus	FilmArray® (+)	4	0	80%	100%	100%	100%	UTC	0.20	0.89 (0.67-1.0)
	FilmArray® (-)	1	248							
	ResPlex™ (+)	2	1	40%	99.5%	67%	99%	99	0.60	0.49 (0.06-0.92)
	ResPlex™ (-)	3	247							
Coronavirus	FilmArray® (+)	21	0	100%	100%	100%	100%	UTC	0.00	1 (1.0-1.0)
	FilmArray® (-)	0	232							
	ResPlex™ (+)	15	1	71%	99.5%	94%	97%	166	0.29	0.80 (0.65-0.94)
	ResPlex™ (-)	6	231							
Human Metapneumovirus	FilmArray® (+)	3	0	100%	100%	100%	100%	UTC	0.00	1 (1.0-1.0)
	FilmArray® (-)	0	250							
	ResPlex™ (+)	3	1	100%	99.5%	75%	100%	250	0.00	0.86 (0.57-1.0)
	ResPlex™ (-)	0	249							
Parainfluenza	FilmArray® (+)	9	0	100%	100%	100%	100%	UTC	0.00	1 (1.0-1.0)
	FilmArray® (-)	0	244							
	ResPlex™ (+)	9	3	100%	98.7%	75%	100%	81	0.00	0.85 (0.68-1.0)
	ResPlex™ (-)	0	241							
Picornaviruses	FilmArray® (+)	31	1	97%	99.5%	97%	100%	214	0.03	0.96 (0.91-1.0)
	FilmArray® (-)	1	220							
	ResPlex™ (+)	22	9	69%	95.9%	71%	95%	17	0.33	0.66 (0.51-0.80)
	ResPlex™ (-)	10	212							
Respiratory Syncytial Virus	FilmArray® (+)	33	2	100%	99.0%	94%	100%	110	0.00	0.97 (0.92-1.0)
	FilmArray® (-)	0	218							
	ResPlex™ (+)	16	0	48%	100%	100%	93%	UTC	0.52	0.62 (0.46-0.78)
	ResPlex™ (-)	17	220							

Sens. - sensitivity
Spec. - specificity
PPV - positive predictive value
NPV - negative predictive value
PLR - positive likelihood ratio
NLR - negative likelihood ratio

Among the 253 samples, 176 (70%) were positive for one or more viral pathogens by either FilmArray® RP or ResPlex™ II. No bacterial pathogens were detected by the FilmArray® RP assay in any of the study samples. Five percent of patients had co-positive infections (see Table 6). The picornaviruses were the most common co-infection virus, with nine co-infections detected. The number and proportion of samples that were positive for each viral pathogen, excluding co-positive infections, are provided in Table 7.

Table 6. Co-positive pathogens detected in

13 patients providing nasal swab specimens

Co-Positive Pathogens	Number Detected
Adenovirus & Picornaviruses	1
Influenza A H1 2009 & Coronavirus	1
Influenza A H3 & Influenza B	1
Influenza A H3 & Picornaviruses	4
Influenza B & Adenovirus	1
Influenza B & Picornaviruses	1
RSV & Picornaviruses	4

Table 7. Infection prevalence by virus type for 242 patients

providing nasal swab specimens who had a single virus detected

Pathogens Detected	# Subjects Testing Positive (%)
Adenovirus	4 (2%)
Coronavirus	19 (8%)
Human Metapneumovirus	3 (1%)
Influenza A	48 (19%)
Influenza A H1 (seasonal)	0 (0%)
Influenza A H1 2009	9 (4%)
Influenza A H3	39 (15%)
Influenza B	30 (12%)
Parainfluenza	9 (4%)
Picornaviruses	25 (10%)
Respiratory Syncytial Virus	27 (11%)
No Pathogen Detected	77 (30%)

Symptom Profiles

By definition, all study patients had a fever of greater than 100°F, with either a cough or sore throat. The symptom questionnaires of influenza positive test result patients reported 80% of patients having a cough and 60% of patients having a sore throat. Other self-reported symptoms that were associated with influenza infection are shown in Figure 1. Excluding chills (which were associated with the presence of fever as required by the case definition), nasal congestion, rhinorrhea, and myalgia were the most common symptoms for patients with a laboratory result of influenza. In general, the proportion of patients reporting any symptom is higher for influenza A infection than it is for influenza B.

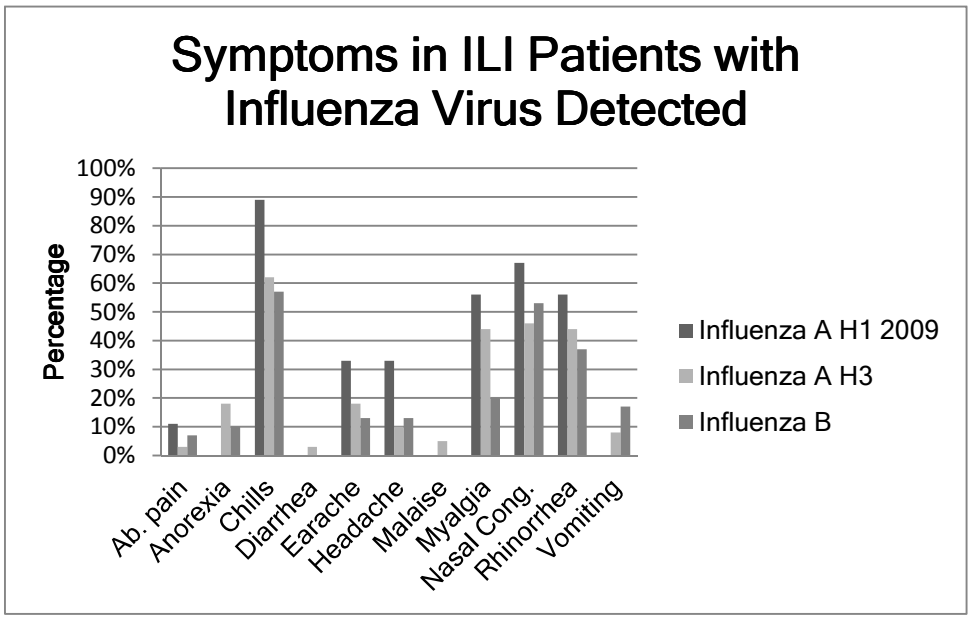

Figure 1. Symptoms in 78 ILI patients with influenza virus detected

Symptoms in ILI patients who had a positive result for a noninfluenza virus are provided in Figures 2 and 3. Among 167 ILI patients who did not have influenza, 77 (46%) did not have a detectable respiratory pathogen by any of the tests we used. As with influenza infection, cough was a more common symptom than sore throat in patients with a noninfluenza virus positive laboratory result (Figure 2). The most common symptoms (again, excluding chills) recorded by patients infected with a noninfluenza virus were nasal congestion and rhinorrhea (Figure 3). Myalgia was also fairly common in patients infected with coronavirus. Headache was infrequently reported among patients who did not have influenza infection, although about 25% of patients infected with influenza A H1 PDM 2009 reported headache.

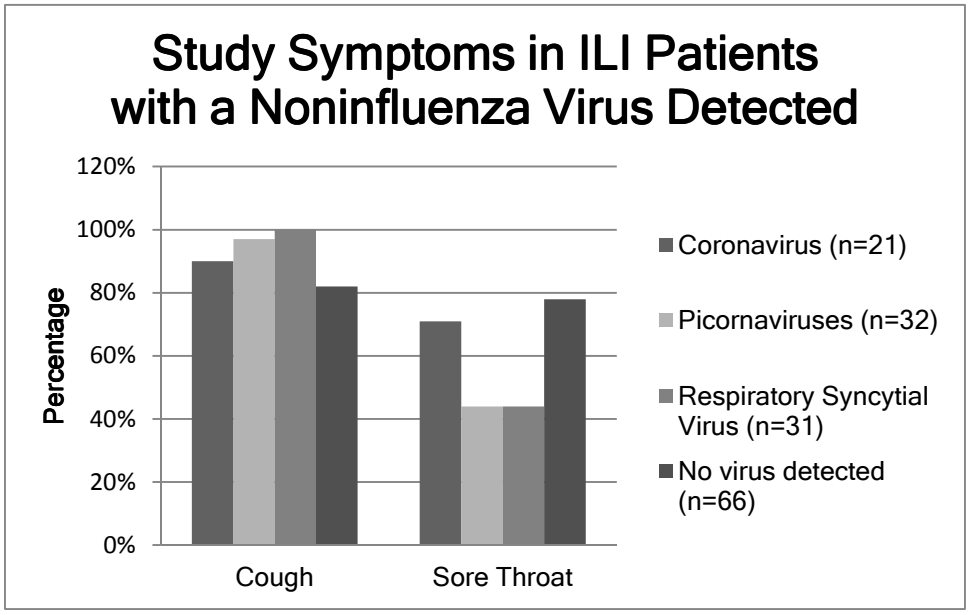

Figure 2. Frequency of cough and sore throat in 167 ILI patients who had a negative PCR test for influenza

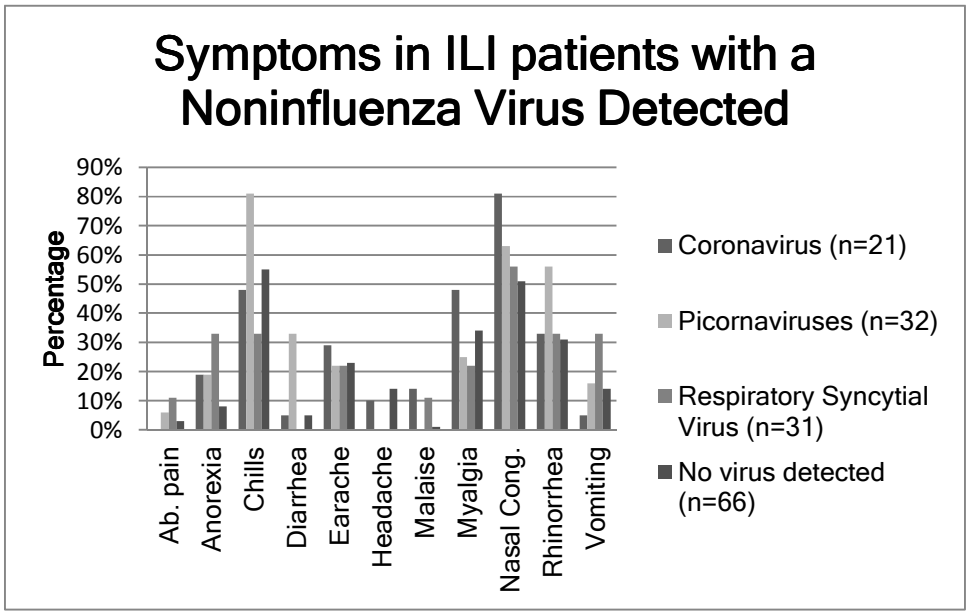

Figure 2. Symptoms in 167 ILI patients who had a negative PCR test for influenza

The self-reported symptoms of the patients with a single virus or no virus detected were evaluated by the Marascuillo procedure to determine if there was a relationship between the virus detected and symptoms. The only symptoms which varied significantly among the viruses were cough and sore throat (data not shown). Patients who were positive for parainfluenza or RSV had a higher proportion reporting cough compared to those with no positive virus result. Patients who were negative for all viruses on the panel had a higher proportion reporting sore throat compared to patients positive for respiratory syncytial virus.

DISCUSSION

Study Findings

Molecular detection techniques have been increasing in use and availability since the year 2000,[18] when real-time PCR assays were routinely introduced into clinical microbiology laboratories. Microarray technology targeting multiple pathogens at a time is a growing field. This study evaluated 2 separate types of microarray technologies: a suspension bead microarray assay (Qiagen ResPlex™ II) and a solid-surface array (Idaho Technology FilmArray® Respiratory Panel).

In this study the Idaho Technology FilmArray® Respiratory Panel had better test characteristics than the Qiagen ResPlex™ II assay. The FilmArray® RP sensitivity ranged from 80%-100%, with concordance expressed as kappa of 0.86 to 1, depending on the virus, versus a sensitivity of 40%-80% for the Qiagen ResPlex™ II, with kappa ranging from 0.49 to 0.86. The FilmArray® RP assay included influenza A virus subtyping and three bacterial pathogens *Bordetella pertussis, Chlamydophila pneumoniae*, and *Mycoplasma pneumonia* not available on the ResPlex™ II.

We found few differences in the proportion of self-reported symptoms by type of viral infection, which suggests that the use of syndromic surveillance is limited in its ability to discriminate between influenza and other types of viral respiratory infection. Patients with an infection caused by parainfluenza or

respiratory syncytial virus are severely ill, and the finding in our study that they reported cough more often than those with a panel-negative result is not unexpected, based on the pathology of their illness. We found that those with a panel-negative result reported sore throat more often than those with respiratory syncytial virus infection. This finding may be a function of the lower sensitivity of PCR tests for picornaviruses,[19] which would increase the possibility of misclassifying a picornavirus as negative on the multiplex panels. Sore throat[20] is a common symptom of picornavirus infection. Respiratory syncytial virus is more common in young children than in other age groups (59% of positive test results were for children under age 5 in our study), which may have meant that parents were unaware of the presence of sore throat. This combination may have resulted in the finding of a significant difference in reported sore throat between those with panel-negative infection and those with respiratory syncytial virus infection.

Strengths and Limitations

Our study of patients with influenza-like-illness in a community-based setting resulted in a large collection of 253 specimens from patients outside of a hospital setting. The specimens were collected by medical assistants at a busy clinic with the collection of study specimens in addition to routine duties. The potential of selection bias was evaluated by comparing the age and sex of the sample to all ILI patients presenting to the clinics participating in the study. No statistically significant differences were found, suggesting that selection bias was minimal. The slightly higher median age in the study population was likely due to

the fact that study participation required a nasal swab and some parents did not want this performed on their children.

In our study, the FilmArray® RP assay was more sensitive than the ResPlex™ II assay. Two differences between the assays may account for this finding. First, in the FilmArray® RP assay each pathogen target has three separate PCR reactions performed and evaluated, compared with one or two targets per pathogen in the ResPlex™ II assay. Second, the FilmArray® RP software analyzes all of the reactions and provides a clear answer for each specimen. The ResPlex™ II assay requires manual data calculations and analysis to determine the positive cut-off threshold value for each virus analyte, resulting in variability in assigning a positive test result each time the test is run. A third possibility had to do with sample quality, because the influenza PCR was run with fresh samples, but all other assays were run with samples that had gone through one freeze-thaw cycle. A study by Luinstra et al.[21] found the viral load of influenza A viruses had low levels of viral quantification loss when stored at 4°C for up to 14 days in viral transport medium; levels of virus started to drop when tested at 21 days. The specimens in our study were stored and transported at 4°C, and all specimens had initial influenza PCR performed within 7 days, and likely had minimal degradation, as suggested by the findings of Luinstra et al. Influenza viruses were found to have a limited reduction in the viral load when limiting the freeze-thaw cycles to less than 4 by Ward et al.[22] The specimen aliquots in our study had a maximum of 2 freeze thaw cycles prior to multiplex testing and confirmatory PCR testing at other labs. While the studies of Luinstra et al. and Ward et al. suggest that the viability of our samples was minimally compromised

prior to testing, it is possible that the FilmArray® RP assay was more robust than the ResPlex™ II assay for frozen samples. Thus, the most appropriate assay might depend on whether a sample was fresh or frozen.

Our study is subject to several limitations. Confirmatory PCR was not performed on all the specimens that were positive for noninfluenza viruses. The gold standard (Table 2) used to calculate the test characteristics was inferred from a combination of assay results. While at least 2 assays were required to be positive to assign a positive result to a sample, a single accepted gold standard such as PCR would have been preferable but was not available in this study.

Comparison with Other Studies

The samples tested as part of this study resulted in 30% of specimens with no pathogen detected. This compares favorably with a large prospective study of acute respiratory infections performed at a regional microbiology laboratory in Vancouver, British Columbia.[23] In the Vancouver study, a total of 1,742 specimens from inpatient and outpatient populations were evaluated, with 32% of specimens negative for all pathogens assayed.

Adenovirus specimens were low in number and presented a challenge in analysis because each multiplex assay detected positive specimens missed by the other assay. The FilmArray® RP has been shown to be less sensitive than real-time PCR adenovirus assays.[24] A study by Pierce et al.[25] found the FilmArray® RP had difficulty detecting some of the adenovirus serotypes when compared with real-time PCR. Neither assay that we used has the accuracy of a real-time PCR adenovirus assay for detecting adenovirus. The number of

coronavirus positive specimens in our study (8%) was similar to the 8.3% detected in a retrospective study in Queensland children in 2004.[26]

The number of RSV infections missed by the ResPlex™ II assay was surprising. Relative to the study-defined gold standard (positive by one of the multiplex assays plus positive by confirmatory PCR), the ResPlex™ II missed 35 specimens compared to the FilmArray® RP which missed 10. The new technology of Idaho Technology FilmArray® RP has 3 primer sets versus the one primer set of the existing usual PCR for detection of RSV. A study by Hayden et al. reported a lack of sensitivity to RSV for ResPlex™ II relative to FilmArray® RP.[27] RSV is a serious illness in the pediatric population under age 2 and the elderly. A false negative RSV result would impact the clinical care of a hospitalized pediatric patient. A previously published ResPlex™ II sensitivity of 73%[28] for RSV is considerably higher than the sensitivity of 48% found in our study. The FilmArray® RP showed a sensitivity of 100% for the detection of RSV in our study, similar to another published study.[29]

Considerations for Clinical Decision-Making

The ResPlex™ II assay requires 55 minutes hands-on time per batch; 1 batch may contain up to 96 specimens and controls, and the total turn-around time is 6 hours per batch. One specimen at a time is run using the FilmArray® RP pouch and instrument; the specimen hands-on time is 5 minutes, and the instrument runs for about 1 hour per specimen.

The initial investment in equipment needed to perform PCR laboratory assays is different between the Qiagen Resplex™ II and the Idaho Technology

FilmArray® RP. The Qiagen ResPlex™ II requires an initial purchase of a nucleic acid extraction instrument, thermocycler instrument, heat block, and a Luminex 100 IS system with the QIAplex MDD software. This total investment is approximately $91,000. The ResPlex™ II assay requires purchasing laboratory consumables in addition to the nucleic acid extraction kits and ResPlex™ II reagent kits. The individual specimen cost for the ResPlex™ II ranges from $74 - $364 depending on the number of specimens per batch. The Idaho Technology FilmArray® RP requires the purchase of a FilmArray® instrument for approximately $45,000. The FilmArray® RP kit contains the laboratory consumables required for testing and the individual specimen cost is $132 per pouch (assay). The ResPlex™ II assay requires a high level of technical expertise to perform the assay and a longer turn-around time of 6 hours after nucleic acid extraction. The FilmArray® RP requires a low level of technical expertise and about 1 hour per specimen. The FilmArray® RP higher cost per pouch could be compensated for by the lower initial investment in instrumentation, lower amount of hand-on time, and lower level of technical expertise needed to run the assay.

Rapid antigen influenza assays are considerably less sensitive than PCR assays for detecting influenza virus infections. Rapid influenza assays are used as point-of-care tests since they are inexpensive and have a quick turn-around-time to result. The low negative predictive value and sensitivity are a problem, with negative results potentially impacting patient treatment decisions due to the inability to distinguish between true and false negatives. The PCR-based assays FilmArray® RP and ResPlex™ II were more sensitive than the rapid antigen assays for influenza. The gold standard influenza assay, a single PCR assay

designed and provided by CDC, detected more positive Influenza A and Influenza B specimens than either multiplex PCR assay. A single PCR assay is typically more sensitive than multiplex assays but lacks the ability to detect additional pathogens at the same time.[30] The multiplex assays detected an additional 102 viruses (40%) in the study patients. The FilmArray® RP performed better than the ResPlex™ II; both are multiplex assays designed to detect multiple pathogens in a single specimen. Additionally, the FilmArray® RP assay also provided Influenza A subtyping results, which has implications for anti-viral treatment decisions[31] by clinicians. The FilmArray® RP has a 1-hour turn-around-time, making it more amenable to point-of-care testing, relative to the ResPlex™ assay which requires both a nucleic acid extraction phase and a 6-hour run time.

Conclusion

ILI symptoms alone are not a good predictor of respiratory tract illness pathogen infection, even during a local outbreak of influenza virus.[32] Rapid and accurate laboratory testing is needed to provide clinicians with laboratory information for diagnosis and treatment decisions. The FilmArray® Respiratory panel provides sensitive, specific, and rapid test results, with better test characteristics than either the Quidel A+B Rapid Antigen test or the Qiagen ResPlex™ II assay. The FilmArray® RP was approved by the United States FDA on May 15, 2012[33] for 21 viral and bacterial targets. The high initial cost of instrumentation for PCR-based laboratory assays currently limits the use of the technology outside of large medical facilities or laboratories. More research is

needed to calculate the true cost of PCR tests in relation to patient treatment decisions in the outpatient setting.

BIBLIOGRAPHY

[1] Diagnosis and treatment of respiratory illness in children and adults. Institute for Clinical Systems Improvement Web site. http://www.icsi.org/respiratory_illness_in_children_and_adults__guideline_/respiratory_illness_in_children_and_adults__guideline__13116.html. Third Addition January 2011. Accessed July 28, 2012.

[2] 2011-2012 Influenza Antiviral Medications: A Summary for Clinicians. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/flu/pdf/professionals/antivirals/clinician-antivirals-2011.pdf. Updated August 30, 2011. Accessed June 30, 2012.

[3] Mundorff MB, Gesteland P, Haddad M, Rolfs R. Syndromic surveillance using chief complaints from urgent-care facilities during the Salt Lake 2002 Olympic Winter Games. *MMWR Surveill Summ*. 2004;53(suppl):254.

[4] Utah 2011-12 Seasonal Influenza Weekly Surveillance Update. Utah Department of Health Bureau of Epidemiology Web site. http://health.utah.gov/epi/diseases/influenza/surveillance/2011-2012_Season/index.html. Accessed July 8, 2012.

[5] QIAamp Viral RNA Mini Handbook [package insert]. Valencia, CA: Qiagen; 2010.

[6] CDC Human Influenza Virus Real-time RT-PCR Detection ad Characterization Panel [package insert]. Atlanta, GA: Centers for Disease Control and Prevention; 2009.

[7] CDC Influenza 2009 A(H1N1)pdm Real-time RT-PCR Detection and Characterization Panel [package insert]. Atlanta, GA: Centers for Disease Control and Prevention; 2010.

[8] ResPlex™ II Handbook [package insert]. Valencia, CA: Qiagen; 2008.

[9] MagnaPure Compact Total Nucleic Acid Kit I [package insert]. Indianapolis, IN: Roche Diagnostics Corporation; 2007.

[10] Balada-Llasat JM, LaRue H, Kelly C, et al. Evaluation of Commercial ResPlex™ II v2.0, MultiCode®-PLx, and xTAG® Respiratory Viral Panels for the Diagnosis of Respiratory Viral Infections in adults. *J Clin Virol.* 2011;50(1):42-45.

[11] FilmArray® Respiratory Pathogen Panel Quick Guide [package insert]. Salt Lake City, UT: Idaho Technology Inc: 2011.

[12] Rand KH, Rampersaud H, Houck HJ. Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG® RVP. *J Clin Microbiol.* 2011;49(7):2449-2453.

[13] Chong S, Louie L, Simor A, et al. Performance comparison of ResPlex™ II and xTag™ RVP Fast for detecting respiratory viruses. Copan Innovations Web site. http://www.copaninnovation.com/studies/index.php?function=advsearch. Published Clinical Virology Symposium April 2010. Accessed July 4, 2012.

[14] VassarStats: Website for Statistical Computation. Chi-Square, Cramer's V, and Lambda Calculator Web site. http://vassarstats.net/index.html. Accessed June 20, 2012.

[15] VassarStats: Website for Statistical Computation. Kappa as a Measure of Concordance in Categorical Sorting Calculator Web site. http://vassarstats.net/kappa.html. Accessed June 20, 2012.

[16]National Institute of Standards and Technology: Engineering Statistics Handbook. Comparing multiple proportions: The Marascuillo procedure. http://www.itl.nist.gov/div898/handbook/prc/section4/prc474.html. Accessed October 3, 2012.

[17] Chartrand C, Leeflang MM, Minion J, Brewer T, Pai M. Accuracy of rapid influenza diagnostic tests: a meta-analysis. *Ann Intern Med.* 2012;156(7):500-511.

[18] Miller MB, Tang YW. Basic concepts of microarrays and potential application in clinical microbiology. *Clin Microbiol Rev.* 2009;22(4):611-633.

[19] McLeish NJ, Witteveldt J, Clasper L, McIntyre C, et al. Development and Assay of RNA Transcripts of Enterovirus Species A to D, Rhinovirus Species A to C, and Human Parechovirus: Assessment of Assay Sensitivity and Specificity of Real-Time Screening and Typing Methods. *J Clin Microbiol.* 2012;50(9):2910-2917.

[20] Monto AS, Bramley TJ, Sarnes M. Development of a predictive index for picornavirus infections. *Clin Infect Dis*. 2003;36(3):253-258.

[21] Luinestra K, Petrich A, Castriciano S, Ackerman M, et al. Evaluation and clinical validation of an alcohol-based medium for preservation and inactivation of respiratory viruses. *J Clin Microbiol.* 2011;49(6):2138-42.

[22] Ward CL, Dempsey MH, Ring CJA, Kempson RE, et al. Design and performance testing of quantitative real-time PCR assays for influenza A and B viral load measurement. *J Clin Virol.* 2004;29(3):179-188.

[23] Brunstein JD, Cline CL, McKinney S, Thomas E. Evidence from multiplex molecular assays for complex multipathogen interactions in acute respiratory infections. *J Clin Microbiol.* 2008;46(1):97-102.

[24] Loeffelholz M, Pong D, Pyles R, et al. Comparison of the FilmArray® respiratory panel and Prodesse® assays for the detection of respiratory pathogens. Idaho Technology Web Site. http://www.idahotech.com/pdfs/Posters/2011/ComparisonofFilmarrayRPandProdesseAssaysforDetectionofRP-2011Pstr.pdf. Published Clinical Virology Symposium April 2011. Accessed July 4, 2012.

[25] Pierce VM, Elkan M, Leet M, McGowan K, et al. Comparision of the Idaho Technology FilmArray System to Real-Time PCR for Detection of Respiratory Pathogens in Children. *J Clin Microbiol.* 2012;50(2):364-371.

[26] Mackay IM, Arden KE, Speicher DJ, et al. Co-circulation of four human coronaviruses (HCoVs) in Queensland children with acute respiratory tract illnesses in 2004. *Viruses* 2012;4(4);637-653.

[27] Hayden RT, Z Gu, Rodriguez A, et al. Comparison of two broadly multiplexed PCR systems for viral detection In clinical respiratory tract specimens from immunocompromised children. *J Clin Virol.* 2012;53(4):308-313.

[28]Haijing L, McCormac MA, Estes RW, et al. Simultaneous detection and high-throughput identification of a panel of RNA viruses causing respiratory tract infections. *J Clin Microbiol.* 2007;45(7):2105-2109.

[29] Babady NE, Mead P, Stiles J, et al. Comparison of the Luminex XTag RVP Fast Assay and the Idaho Technology FilmArray® RP Assay for detection of respiratory viruses in pediatric patients at a cancer hospital. *J Clin Microbiol.* 2012;50(7):2282-2288.

[30] Arens MQ, Buller RS, Rankin A, et al. Comparison of the Eragen Multi-Code Respiratory Virus Panel with conventional viral testing and real-time multiplex PCR assays for detection of respiratory viruses. *J Clin Microbiol.* 2010;48(7):22387-2395.

[31] 2011-2012 Influenza Antiviral Medications: A Summary for Clinicians. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/flu/pdf/professionals/antivirals/clinician-antivirals-2011.pdf. Updated August 30, 2011. Accessed June 30, 2012.

[32] Woolpert T, Brodine S, Lemus H, et al. Determination of clinical and demographic predictors of laboratory-confirmed influenza with subtype analysis. [published online ahead of print June 7, 2012]. *BMC Infect Dis.* doi:10.1186/1471-2334-12-129.

[33] Idaho Technology Receives FDA 510(k) Clearance for Expanded FilmArray® Respiratory Panel Press Release. Idaho Technology Website. http://www.idahotechnology.com/pdfs/mediakit/PRESS%20RELEASE%20-%20FDA%20Clears%20FA%20RP.pdf. Accessed July 7, 2012.